VITASSAY

FOB

-Turbidimetric Assay-

Rapid test for the quantitative detection of human hemoglobin in human stool samples.

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CE IVD EN

Vitassay

For professional *in vitro* diagnostic use only. Professional trained in Turbidimetric techniques.

INTENDED USE

Vitassay FOB –Turbidimetric Assay- is a rapid Turbidimetric assay for the quantitative detection of human hemoglobin in human stool samples.

Simple, non-invasive and highly sensitivity assay for the detection of human hemoglobin in feces that helps in the search for gastrointestinal bleeding problems. This product has been optimized for several automated analyzer.

INTRODUCTION

The increased amount of hemoglobin (hHb) in feces is the main symptoms of illnesses accompanied by hemorrhagic lesions in the digestive tract, particularly in the lower digestive tract. Therefore, measuring the amount of hemoglobin in feces is an effective method for the early detection and treatment of colon cancer and other diseases of the lower digestive tract that are accompanied by hemorrhaging.

PRINCIPLE

Vitassay FOB –Turbidimetric Assay- is a quantitative turbidimetric assay for the detection of human hemoglobin in human solid stool samples.

FOB Turbidimetric Assay is based on antigen-antibody agglutination reactions between the antigen contained in the sample and the antibodies anti-antigen coated on polystyrene latex particles.

Such agglutination is measured as an increase in absorbance proportional to the quantity of antigen contained in the sample.

The use of two external controls, Control 1 and Control 2, is used to verify that the test is working properly.

PRECAUTIONS

- For professional *in vitro* use only.
- Do not use after expiration date.
- Do not use the test if its primary containers are damaged.
- Specimens should be considered as potentially hazardous and handle in the same manner as an infectious agent. Avoid contamination errors, follow proper work procedure.
- The reagents after use should be discarded in a proper biohazard container after testing.
- Reagents contain preservatives. Avoid any contact with the skin or mucous membrane. Consult safety data sheet, available on request.
- Components provided in the kit are approved for use with the **Vitassay FOB-Turbidimetric Assay-**. Do not use any other commercial component.
- Follow Good Laboratory Practices, wear protective clothing, use disposal gloves, goggles and mask. Do not use any other commercial kit component.
- If measure range is exceeding, use the sample diluent to dilute the sample and repeat the assay again.
- Read and follow up the instructions for use provided in the kit.
- Prepare and adjust the analyzer before starting measurements.

STORAGE AND STABILITY

Store as packaged in the original primary container, the reagents should be preserved at refrigerated temperature (2-8ºC/36-46.4ºF), the sample diluent could be preserved refrigerated or at room temperature (2-30ºC/35.6-86ºF).

The product is stable until the expiration date printed on the label, if they have been preserved under the recommended conditions.

Do not freeze and keep away from the sunlight.

MATERIALS

MATERIAL PROVIDED	MATERIAL REQUIRED BUT NOT PROVIDED
▪ Reagent R1 ▪ Reagent R2 ▪ Calibrator 0 ▪ Calibrator 1 ▪ Calibrator 2 ▪ Calibrator 3 ▪ Calibrator 4 ▪ Calibrator 5 ▪ Control 1 ▪ Control 2 ▪ Vials with diluent for the sample dilution. ▪ Instruction for use.	▪ Specimen collection container. ▪ Disposable gloves. ▪ Automated analyzer. ▪ Vortex. ▪ Microtubes (analyser vial).

SPECIMEN COLLECTION

Collect sufficient quantity of feces: 1-2 g or mL for liquid samples. Stool should be collected in clean and dry containers.

Samples can be stored in the refrigerator (2-8ºC/36-46.4ºF) for 2 days prior to testing. For longer storage, maximum 6 months, the specimen must be kept frozen at -20ºC (-4ºF). Samples must be brought to room temperature before testing. Homogenize the sample as thoroughly as possible prior to preparation.

SPECIMEN PREPARATION

1. Label the vial with the patients´s identification.

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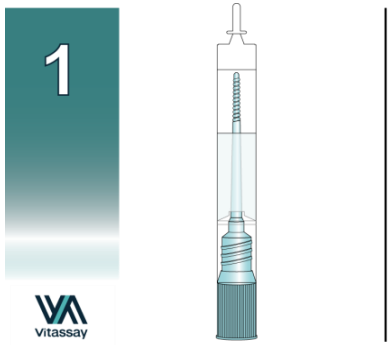
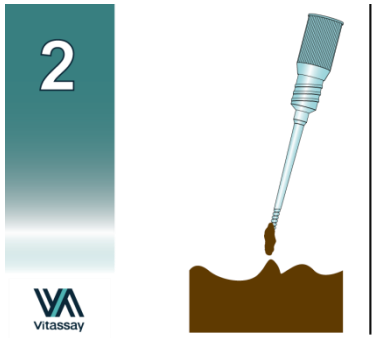
2. Open the cap of the vial with diluent for sample dilution (figure1).
3. Use the stick to collect sufficient sample quantity. For solid stool, insert the stick in 4 different areas of the stool sample (figure 2), and add it into the vial with diluent for sample dilution. For liquid stool, take approx. 15 µL of the sample using a micropipette and transfer it into the vial for the sample dilution.
4. Close the cap of the vial with the diluent and sample, shake the vial in order to assure good sample dispersion, using a vortex during 1 minute (figura 3). The sample dilution vial with dilute sample can be stored in a range of temperatures (2-8ºC / 35.6-46.4) for 5 days in the refrigerator prior the testing.
5. Take the specimen collection vial with the sample diluted, cut the end of the cap and dispense 20 drops of the sample diluted (figure 4) into a analyser vial (microtube). This vial must be compatible with the analyzer.

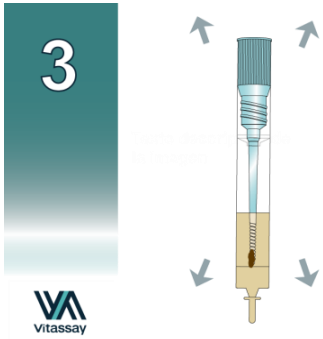
Note: Do not use the sample vials directly in the analyzer.



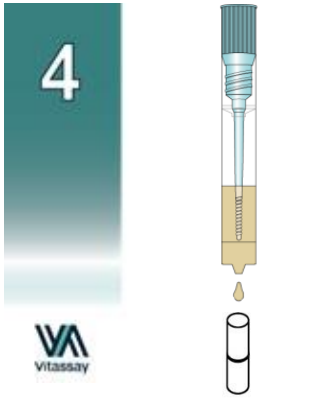
Vial for sample dilution.



Insert the stick in 4 different areas of the stool.



Put the sample into the vial, close the vial and shake for a good dispersion of the samples (vortex until the sample is fully resuspended max.1 minute).



Dispense 20 drops into a analyser vial (microtube).

PROCEDURE

Allow reagents and solid stool to reach room temperature (15-30ºC) prior the testing. Reagent R1 y Reagent R2 are ready to use.

Calibration curve

For calibration only use FOB Calibrator: Cal0, Cal1, Cal2, Cal3, Cal4 and Cal5. Contain human hemoglobin at diferent concentrations indicated on the label of each of the vials.

	Reference	Calibrator 1	Calibrator 2	Calibrator 3	Calibrator 4	Calibrator 5
Con.	0 ng/mL	50 ng/mL	100 ng/mL	250 ng/mL	750 ng/mL	1500 ng/mL
Vol.	300 µL	300 µL	300 µL	300 µL	300 µL	300 µL

Reagents are ready to use. The frequency in the realization of the calibration curve must be established by the end user in the function of the criteria esblished for the clinical laboratory.

Note: See section quality control.

Analytical procedure

Measure range: 20 – 1000 ng hHb/mL.

Procedure	Steps	
R1 addition	200 µL	0 s
Sample addition	20 µL	10 s
R2 addition	55 µL	300 s
Blank measure	505 nm – 800 nm	310 s
Mainly measure	505 nm – 800 nm	610 s

INTERPRETATION OF RESULTS

Cut-off must be fixed by the clinical laboratory.

Recommended cut-off values: 5 µg de hHb/g of stool (50 ng/mL) for diagnostics procedures and 20 µg de hHb/g of stool (200 ng/mL) for screening procedures.

Values higher than the cut-off determine the abnormal presence of human hamoglobin (hHb) in stool samples.

QUALITY CONTROL

FOB C1 & C2 Controls are ready to use. Allow controls to reach room temperature (15-30ºC) prior to testing.

FOB Control 1: is liquid control at a certain concentration of recombinant human hemoglobin (lower than Control 2). Concentration is indicated on the label of the vial.

FOB Control 2: is liquid control at a certain concentration of recombinant human hemoglobin (higher than Control 1). Concentration is indicated on the label of the vial.

The use of controls at two different concentrations is recommended to verify test precision.

If the obtain results are out of the tolerance range, the equipment, the reagents or the technique must be reviewed. If the problems persists, stop using the reagents and contact your distribuitor.

LIMITATIONS

- **Vitassay FOB -Turbidimetric Assay** should be only used in human solid stool samples.
- The quality of **Vitassay FOB -Turbidimetric Assay-** depends on the quality of the sample; Proper fecal specimens must be obtained.
- Positive results determine the presence of human hemoglobin in fecal samples; nevertheless, it can be due to several causes, besides colorectal bleeding, (hemorrhoids, blood in urine or stomach irritations). A positive result should be followed up with additional diagnostic procedures to determine the exact cause and source of the blood in the stool.
- If the test result is negative and the clinical symptoms or situation persist, it is recommended to perform another detection method. Negative results do not exclude hemorrhages caused by some polyps or colorectal cancer, as they can cause bleeding intermittently or not cause them during any phase of the disease.

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In addition, blood may not have been distributed uniformly in the stool sample.

- Patients should not collect samples during their menstrual period, if they have bleeding hemorrhoids, blood in urine or if they have strained during bowel movement.
- This test may be less sensitive for the detection of upper gastrointestinal bleeding.
- This test has not been validated in patients with hemoglobinopathies.
- All results must be interpreted along with the rest of the clinical information and other results obtained in the laboratory by a specialist doctor.

EXPECTED VALUES

The cut-off value commonly established is 5.1 µg of hemoglobin per g of feces. However, this value is not established in all studies. The Spanish CRC programs establish a value of 20 µg/g to study the convenience of performing colonoscopies. Therefore, those tests that are capable of quantifying in the range between 5.1 and 20 µg/g of feces are interesting. It should be noted that the conversion between µg/g to ng/mL is dependent on the sampling process of each product, in this case it must be multiplied by a factor of 10.

PERFORMANCE CHARACTERISTICS

Analytical sensitivity:

Detection limit: 15 ng hHb/mL.

Cut-off value of Vitassay FOB-Turbidimetric Assay-: 50-200 ng hHb/mL (:10 dilution factor, 5-20 µg hHb/g of sample)

Hemoglobin concentration values greater than 200 ng hHb/mL are indicators of bleeding from gastrointestinal tract.

Prozone:

Lower concentrations of 10 µg of hHb/mL of stool do not show prozone effect and no false negative results have been observed.

Within-Run Precision:

	Low (20 µg/g)	Media (80 µg/g)	High (250 µg/g)
N	20	20	20
Media (µg/g)	22.1	82.7	255.9
DS (µg/g)	1.3	4.9	9.1
CV (%)	6.1	5.9	3.5

Cross reactivity:

No cross reactivity was detected against other fecal markers that are occasionally present in feces:

Bovine lactoferrin	*None*	*Bovine haemoglobin*	*Concentration lower than 1 µg/mL*
Human calprotectin	*None*	*Pig haemoglobin*	*Concentration lower than 2.5 µg/mL*
Human lactoferrin	*None*		
Human transferrin	*None*		

REFERENCES

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3. Fenocchi E, Martínez L, Tolve J, Montano D, Rondán M, Parra-Blanco A, Eishi Y. Screening for colorectal cancer in Uruguay with an immunochemical faecal occult blood test. Eur J Cancer Prev. 2006 Oct;15(5):384-90.

SYMBOLS FOR IVD COMPONENTS AND REAGENTS

IVD	*in vitro* diagnostic device		Keep dry
	Consult instructions for use		Temperature limitation
	Use by		Manufacturer
LOT	Batch code	Σ n	Contains sufficient for <n> test
DIL	Sample diluent	REF	Catalogue number

ADAPTED EQUIPMENT

- Biolis i24 (Tokio Boeki)
- BS200 (Mindray)
- Architect 4000/8000 (Abbott)
- Vitros 5600 (Ortho)
- ChemwellT (Awareness)
- A15 (Biosystems)
- Cobas c501 (Roche)

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